REMARKS

Reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Claims 1-24 have been cancelled without prejudice or disclaimer. New claims 25-33, all of which are fully supported in the as-filed application, have been added.

It is respectfully submitted that the issues raised by the Examiner in the §112, second paragraph rejection have all been addressed. Specifically, all of the new claims are product-by-process in their format.

The "Use" claims have been deleted.

The phrase "are chosen in the group of" has been replaced by "selected from the group consisting of" as suggested by the Examiner.

The phrase "active principles of vegetable origin" has been deleted. The phrase "said fish are in the early stages of growth" has been deleted.

Food compositions have been limited to those comprising microorganisms filled with antibiotics, anti-inflammatory agents, anti-bacterial agents, anti-viral agents, anti-fungal agents, antiparasitic agents, vaccines and nutritional substances (new claim 30) or to those comprising microorganisms filled with the antibiotic oxytetracycline or the antibacterial sodium sulphadimethoxin (claim 32) or to those comprising microorganisms including a nutritional substance selected from the group consisting of sodium quercetin, catechin, isocatechin, aliphatic polyalcohols, polyphenols, flavans, cyanins, resveratrol and hyperic acid (claim 33).

In view of the foregoing, the rejection under §112, second paragraph, has been overcome, and its withdrawal is respectfully solicited.

Claims 1 and 4-7 stand rejected under §102(b) as anticipated by Pannell,

U.S. 5,288,632 with evidence provided by Gruenwald et al., (PDR for Herbal Medicine, 1998, pgs 836-839. This rejection is respectfully traversed.

Before addressing the substance of the §102(b) rejection, Applicant wishes to point out to the Examiner that the new set of claims no longer recite "microorganisms containing vitamins or bioflavonoids.

In addition, Applicant also wishes to bring to the attention of the Examiner that the novelty of the claimed invention, and the manner in which it distinguishes over the teachings of the Pannell reference, is based upon the process for obtaining the microorganisms.

In fact, the distinguishing feature of the microorganisms prepared according to the claimed invention, which also represents a major distinguishing feature over both the Pannell and Sagar references, resides in the fact that in the claimed invention, the microorganisms *are first emptied by treatment in a hypertonic solution*, and only then, after they are first emptied, are they then filled-up with the pharmacologically active substance according to the method of the claimed invention. Therefore, the microorganisms claimed herein are *empty cell walls which are thereafter filled-up with the active substance.*

Accordingly, the fact that the microorganisms are emptied first, really makes the difference and distinguishes in terms of the final product, since the microorganisms of the claimed invention are devoid of any of their original contents and are only filled-up with the active substance.

This peculiarity is particularly stressed at p. 7 of the specification, in the paragraph reading, as follows:

"The suspension obtained is stirred at a temperature ranging from 2 to 40°C, preferably at 25°C, for a period ranging from 2 hours to 4 days, preferably 16 hours, to complete emptying of the microorganisms in such a way that the endocellular content is extruded into the hypertonic medium.

The so treated microorganisms are reduced to the cell walls alone and are smaller than the alive microorganisms; they presents enlarged membrane pores. Empty cell walls may be separated from the endocellular mass by means of techniques known in the state of the art, such as filtration or centrifugation.

The control to ensure that the endocellular mass has been removed to leave just the cell walls may be conveniently performed by morphological microscopic evaluation of the cell walls, which must appear smaller and shrivelled.

The suspension medium (i.e. the hypertonic buffer and the squeezed out endocellular content) can be separated from the microorganisms cell walls, by centrifugation at 2.000-12.000 r.p.m., preferably 4.500 r.p.m (corresponding to about 4600 R.C.F.)".

By contrast, Pannell discloses that encapsulation of different substances in fungi or yeast is obtained by simple mixing of the material to be encapsulated together with the yeast. In Pannell, the microorganisms are not emptied first, as the substance to be incorporated simply diffuses into them, and, therefore, they are not devoid of their own content of nucleic acids and proteins.

This is the major distinction between the claimed invention and the disclosure of Pannell.

In accordance with the foregoing and with the aim of better pinpointing this feature, the microorganisms have been now claimed as product-by-process.

Since the claims now distinguish over the disclosure of Pannell, withdrawal of the §102(b) rejection is respectfully solicited since a *prima facie* case of anticipation has not been established.

The Examiner has rejected claims 1-15 under §103(a) over the combination of Pannell with evidence by Gruenwald in view of Sagar (WO94/22572). This rejection is respectfully traversed.

The same considerations which applied to the Pannell reference discussed above also apply to the disclosure of Sagar. In Sagar, the microorganism is prepared by mixing with the substance to be incorporated dissolved in a solvent, such as ammonia (see page 3 of WO94/22572), according to a process which is much closer to the process disclosed by Pannell than to the claimed process, and which does not disclose, suggest or even intimate the step of emptying the microorganism.

The novel feature of the microorganism produced according to the invention and defined by its process of preparation also results in the following advantages:

1) the microorganism is devoid of the unnecessary and undesirable microorganic content (mainly nucleic acids and proteins etc); and
2) the microorganism is also inactivated and, therefore, a further treatment to achieve their inactivation, such as a thermal treatment, is only optional.

As far as 1) is concerned, Pannell discloses the encapsulation of different substances in fungi or yeast by simply mixing the solubilized material to be encapsulated with the

yeast. Microorganisms are not emptied first. The substances simply diffuse into the microbial cell wall (see col. 2, l. 29-47).

The same applies to the process described by Sagar wherein the microorganism is prepared by mixing with the substance to be incorporated dissolved in a solvent, such as ammonia (see page 3) and as discussed above.

The simple mixing performed in Sagar's process is not a guarantee of microorganism inactivation and, in this regard, the use of strains which have already been inactivated and which are unable to propagate is in fact suggested by Pannell (see col. 2, line 60-66). However, Pannell does not teach that for the inactivation of microorganisms they need to be first emptied, nor is this step even suggested among the different means for microorganism preparation.

Moreover, according to Pannell, even though it may occur that the microorganism is eventually inactivated (See col. 4, l. 38-39), where it states "*Usually the microbe will be killed as a result of encapsulation*", it still contains and carries its own content of nucleic acids and proteins.

This aspect may be critical in particular when microorganisms are prepared for feed consumption, as almost 20% of the nitrogen in yeast is in the form of nucleic acids and "*.....nucleic acids can cause problems if over-fed because excessive nucleic acid intake results in elevated uric acid levels in the blood. High level of uric acid tend to crystallize in the joints.... and this can cause gout and arthtritis or even renal stones*" as depicted in a booklet explaining the nutritional value of yeast preparations and which is enclosed for the Examiner's convenience (see p. 3, highlighted paragraph) (http://www.diamondv.com/articles/booklet/booklet.html).

By contrast, the microorganisms obtained according to the claimed process of the present invention are devoid of their own content of nucleic acids and proteins. This represents not only a way of distinguishing them with certainty from the prior art products, but also provides an undisclosed and not previously suggested means of administering the active principle, which avoids carrying along any potentially harmful or adverse effects.

It derives from the foregoing that the microorganism obtainable according to the process of the present invention is not only novel but also unobvious.

As far as 2) above is concerned, the controls performed by Applicant demonstrate that there is no residual microbial growth in any of the ten (10) experiments performed to produce yeast filled with different substances by the hypertonic/hypotonic treatment in accordance with the claimed invention. These results are shown in the tables at pages: 16 (step VI Example 1), 21 (step VII Example 2), 26 (step VII Example 3), 31 (step VII Example 4), 36 (step VII Example 5), 37 (step VII Example 6), 40 (step VI Example 7), 44 (step VI Examples 8-9), or has been determined at page 45, in example10.

The controls demonstrate, therefore, that 100% of the treatment ends up with inactivation of the microorganism. The controls performed by the Applicant confirm without any doubt that microorganism inactivation is achieved as a result of the treatment. This experimentally proven certainty is more than the "usual" statement of killing given by the prior art reference.

In conclusion, while one of ordinary skill in the art may be motivated to incorporate different nutrients or pharamaceutical substances into microorganisms according to the teaching of Pannell, there is no teaching or suggestion of the claimed process as a whole, and the advantages accruing and flowing from the microorganisms obtained at the conclusion of the process, from the combination of Pannell and Sagar references.

Accordingly, the rejection under §103(a) having been overcome, its withdrawal is solicited.

The issuance of a Notice of Allowance is requested.

Please charge any fees which may be due to our Deposit Account No. 01-0035.

Respectfully submitted,

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Yeast Products in the Feed Industry
A Practical Guide for Feed Professionals

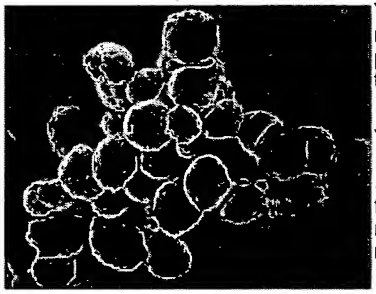
Charlie W. Stone

Introduction

The purpose of this booklet is to provide both a semi-technical and non-technical understanding of the various yeast products offered t and used by, the feed industry.

It does not deal with animal research and product efficacy, but merely describes what the products are and the benefits they are purpor to provide. Efficacy in the animal is too complex a subject to deal with in this short booklet. The hope is that the reader will get a better understanding of what the various yeast products are, so that he or she can make educated decisions about the various products, base on the knowledge of what they are and the research data presented by the supplier of the product. Feed industry professionals can not afford to take the attitude that "yeast-is-yeast", because there are significant differences in the various yeast products. There are both biochemical differences, as well as functionality differences, and each product should be used for its own unique properties. Not all yea products are the same.

Yeast -- An Overview



Yeasts have been fed to animals for more than a hundred years, either in the form of yeast fermente mash produced on the farm, yeast by-products from breweries or distilleries, or commercial yeast products specifically produced for animal feeding. Although yeast feeding has been around a long tim there is confusion throughout the industry concerning what the various yeast products really are.

Yeasts are microscopic fungi -- single-cell organisms of the plant kingdom which are generally about 10 microns in size. They are given Latin names which represent their genus and species (e.g., *Saccharomyces cerevisiae* or *Candida utilis*). The species differ from each other by: where they are found, their cellular morphology or shape, how they metabolize different substrates, and how they reproduce. While there are nearly 50,000 species of fungi, there are only 60 different genera of yeast representing about 500 different species.

Yeasts are abundant throughout the environment. They can be found on cereal grains, grain by-products, silages, hays and are even present in the soil and water. Our laboratory has found that various feed ingredients contain anywhere from a few thousand (10^3) live y cells per gram to over a million (10^6) per gram. Several species have proven very beneficial to man, while a few imperfect yeasts are known to be pathogenic. But, most yeasts are benign saprophytes and have proven neither useful nor harmful to man or animal.

Very few species of yeast are used commercially. *Saccharomyces cerevisiae*, also known as "bakers yeast", is one of the most widely commercialized species. Selected strains of this yeast are used by breweries to make beer and ale, distilleries to make distilled spirits industrial alcohol, and wineries to make wine. *Candida utilis* (formerly classified as *Torulopsis utilis*) is the yeast known as "Torula Yeas This yeast is important because it can utilize the pentose sugars from processed wood pulp used in making paper. A third useful yeast i *Kluyveromyces marxianus*. This is the "Whey Yeast", which can utilize milk sugar or lactose as a substrate.

Yeasts are "facultative anaerobes" which means that they can survive and grow with or without oxygen. Yeast propagation is an aerobi processes where the yeast converts oxygen and sugar, through oxidative metabolism, into carbon dioxide and usable free energy for efficient yeast cell growth. However, the production of alcoholic beverages (beer, wine, whiskey, etc.) and industrial alcohol are anaero processes. Anaerobic fermentation is much less efficient, resulting in considerable "metabolic by-product" in the form of ethyl alcohol. T yeast ferments simple sugars into ethanol and carbon dioxide and the yeast grows very slowly. To optimize ethanol production, the fermentation process is carried out without oxygen being present; but, to maximize yeast cell growth, an abundance of oxygen is provid in the form of air.

Assimilation patterns of various sugars by diff rent yeast species.

	Yeast Species		
	Saccharomyces cerevisiae	*Kluyveromyces marxianus*	*Candida utilis*
	(Bakers)	(Whey)	(Torula)
Glucose	+	+	+
Sucrose	+	-	-
Lactose	-	+	-
Xylose	-	-	+

Adapted from Reed and Nagodawithana, 1991.

In the early days, fermentations were carried out by seeding bread dough, gra must or corn mash with retained portions from a previous fermentation. Our ancestors always retained a portion of a fermenting bread dough for mixing wit fresh dough the following day. This yeast dough was called the "starter dough The live yeast was carried from dough-to-dough in a perpetual cycle. If the sta was lost or it turned sour due to bacterial contamination, a new seed could be prepared by moistening flour and waiting for a spontaneous fermentation to oc or by borrowing some starter from a neighbor and initiating a new starter fermentation.

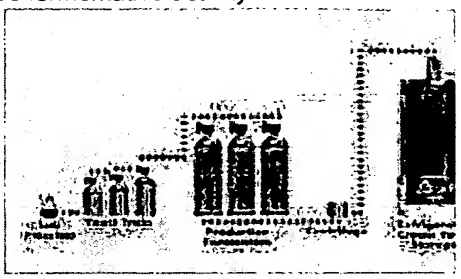
Today, pure cultures of yeast are grown specifically for breweries, wineries, distilleries, bakeries and home use. Commercial or proprietary yeasts are use industrially for the production of all yeast-raised baked goods and alcoholic beverages. Although a few wineries still use the natural yeast found on the gra to spontaneously ferment their wines, most wineries now depend on pure cultures of specific yeast strains to make consistent proprieta wines.

Viabl Yeast Products

Active dry yeast (95% dry matter) is the predominant viable yeast available to the feed industry. Although wet yeast cake (30% solids), to a lesser extent yeast cream (18-20% solids), are used extensively by the bakery trade, active dry yeast is the form of viable yeast us

The production of active dry yeast is accomplished using submerged culture bioreactors or aerobic ferment The objective is to feed the yeast nutrients (oxygen, nitrogen, carbohydrate) and allow them to reproduce a form new generations of yeast cells. They reproduce using "budding", where a daughter cell evolves from t mother cell, forming a bud, and when the daughter is mature, they will both reproduce. Thus, their growth is exponential.

For example, one cell becomes two, two becomes four, four becomes eight, and so on. After the effective concentration of yeast is achieved, the growth-broth is centrifuged to form a yeast cream, filtered through filter presses to make yeast c formed into wet yeast-noodles, and then dried at temperatures which will not destroy the yeast's fermentative activity.



Active dry yeast consists of pure, dried yeast cells with viability counts ranging from 15-25 billion live yeast cells or colony forming units (cfu) per gram. It is marketed in three physical forms, depending on the process used to dry the yeast: *tunnel dried yeast* which is a granular powder, *fluid-bed dried yeast* (also known as Instant or Quick Rise yeast) which looks like small torpedoes, and *rotolouver dried yeast* which looks like small spheres or balls. In the United States, tunnel dried yeast and fluid-bed dried yeast are most common, while rotolouver dried yeast is most prevalent in Europe and Latin America. The fluid-bed drying process is becoming more popular, because it causes less damage to the yeast cells, resulting in better leavening properties in the yeast.

Active dry yeast is showing up more frequently in the feed industry, not as the pure product, but in the form of diluted yeast products ha a wide range of yeast viability counts. This is one reason there is so much confusion about live yeast products. For example, a live yea product guaranteeing 5 billion cfu per gram of live yeast count contains only 20-25% active dry yeast, which generally has about 20 billi cfu per gram. The remainder of the product consists of cheaper diluents like rice hulls or distillers solubles. Active dry yeast generally c less than 7.5 cents per billion cfu per gram of yeast viability, while the diluted products currently on the market sell for 10-15 times as m per billion. Therefore, it is important that the nutritionist and purchasing agent know what they are buying and how their cost per billion compares to commercial active dry yeast.

Storage stability of active dry yeast in different atmospheres *	
Atmosphere	Relative Activity
Air	43.3%
Vacuum	70.3%
Nitrogen	79.5%
Carbon Dioxide	81.9%

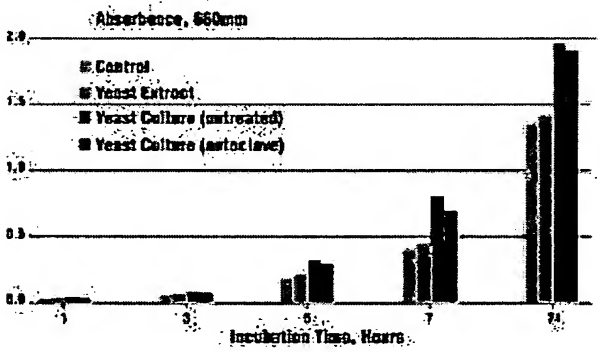
* After three days storage at 131° F (55° C). Adapted from Reed and Nagodawithana, 1991.

The stability of active dry yeast is dependent upon how the product is packaged ... whether o not oxygen is present. Yeast packaged in vacuum or inert gas has much greater stability than yeast packaged in air. Commercially, most active dry bakers yeast is packaged in either a vacuum pack or purged with nitrogen gas. The presence of air or oxygen has a dramatic effe on fermentative activity.

Effects of Pelleting on Yeast Viability

Significant confusion exists in the feed compounding industry concerning the effects of pelleti live-cell yeast products and yeast cultures. This confusion stems from the lack of differentiati between the two products and a misrepresentation of what pelleting does to the live yeast cel

Yeast cultures have been manufactured in the United States for more than 50 years, but the last decade has witnessed a proliferation commercial live-cell yeast products being touted as concentrated, high-cell count "yeast cultures". These products are not, however, fermented yeast cultures, but rather mixtures of dried viable yeast (active dry yeast) diluted with a carrier. Unfortunately, this has cause misunderstanding of what true yeast cultures are and how they differ from live-cell yeast products.



True yeast cultures are complex fermented products containing the yeast and metabolic by-products produced by the yeast during fermentation. Yeast cultu are not fed as a source of live or viable yeast cells, but as a nutrition supplem to provide undefined fermentation factors, which are recognized to stimulate bacterial growth in the digestive tract. These fermentation factors, sometimes referred to as "nutrilites", appear to be heat stable and are not significantly affected by high temperatures or pelleting. This heat stability is illustrated in t figure above, demonstrating that even autoclaving has minimal affect on yeas culture's ability to enhance bacterial growth.

Live-cell yeast products, on the other hand, consist of viable active dry yeast blended with a diluent to provide a specified number of live yeast cells. Active yeast is defined as pure dried yeast (without fillers or diluents) containing not l than 15 billion (1.5×10^{10}) live yeast cells per gram. Thus, a live-cell product claiming 5 billion cells per gram would consist of 20-25% active dry yeast, with the remainder being carrier ingredients like distillers solubles or rice hulls. These definitions help differentiate active dry yeast and true yeast cultures; the first being a source of viable yeas cells and the second being a fermented culture. Since the live-cell yeast products are made with active dry yeast and marketed as a so of viable yeast cells, the question of whether or not pelleting destroys their activity is a major issue to the feed manufacturer or nutritioni using them in pelleted products.

The normal pelleting process utilizes steam to condition the feed for proper extrusion through the pellet die. It is generally accepted that steam is one of the most effective methods for killing microorganisms (steam is used in autoclaving for sterilization). Moist heat denatur the enzyme systems within the organism, destroying their metabolic activity and inhibiting life processes. The higher the temperature, t more complete and irreversible the process. It is also well documented that dry heat is much less destructive to yeast viability and enzy activity than moist heat. Therefore, heating dry yeast without moisture present is much less detrimental to the yeast cell than when stea used.

To determine whether or not pelleting adversely affects yeast viability and/or fermentative activity, there are three possible approaches:

Yeast Plate Counts:

The plate count technique is the most accurate method, because it determines the number of actual live, viable yeast cells in the produ before and after pelleting. The method is performed by serially diluting a suspension of the yeast-containing product in water and platin the different dilutions on nutrient media to allow the yeast cells to grow and multiply. After about three days, each place on the nutrient where a single yeast cell was deposited will grow up into a colony consisting of thousands of yeast cells and the colonies become visibl the naked eye. Th numb r of colonies are counted on each plate and the live-yeast count of the product tested is computed and repor as colony forming units per gram (CFUs per gram).

pasteurization and sterilization with heat. Heat can denature the metabolic enzyme systems of the yeast cell, which not only prevent metabolic activity, but reproduction and life itself. Because of this relationship, if pelleting-heat prevents the yeast cell from reproducing petri dish, it most likely also destroys the yeast cell's ability to carry out other metabolic functions as well. This is why the plate count method is the method of choice to test the effects of pelleting on yeast activity – it is a well established technique and is quite accurate, plus it allows the use of selective growth media to prevent interference from other non-yeast microorganisms in the feed mixture.

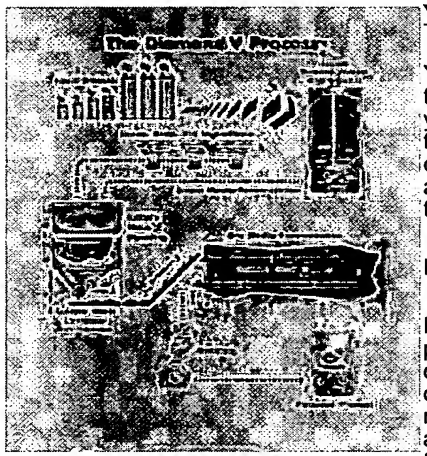
In evaluating losses in yeast viability, it is important not to use logarithms, but actual numbers, to express viability counts, because logarithms can be misleading. For example, if a feed contains 5 million (5.0×10^6) cfu per gram before pelleting and only 50 thousand ($\times 10^4$) after pelleting, that represents an actual 99% loss in viability. However, if the cfu numbers are converted to $\log_{10}$ logarithms, the numbers would be represented as 6.699 before pelleting and 4.699 after pelleting which makes it look like only a 30% loss. This techni has been used in the past to imply that pelleting has less effect on yeast viability than it really does. So, make sure you understand ho cfu counts from an experiment are being presented so you can make an accurate interpretation.

Metabolic Activity:

This method might be an alternative to the plate count method, since it deals directly with measuring the end-products of metabolic acti or the disappearance of substrate. However, it has some important draw-backs. There might be considerable interference from other microorganisms, especially bacteria. Many thermophilic bacteria could survive pelleting and their metabolic by-products could interfere the interpretation. Also, the feed being pelleted would probably need to have higher than normal inclusion of yeast so that their metabol could be detected following a short fermentation period. A simple manometric apparatus could be used to measure carbon dioxide volu while more complex analytical procedures would be necessary for monitoring glucose disappearance or ethanol production.

Methylene Blue Staining:

This method is not recommended, even though it is a very quick and simple method. Our laboratory has tried to correlate this quick met with actual yeast viability and fermentative activity many times, with little success. This technique seems to correlate well when the yea populations are healthy and yeast viability is reduced by natural aging and autolytic death. However, it is not a reliable method when ye cells have been subjected to heat, because it tends to give an over-estimation of viable yeast counts. Methylene blue staining depends destruction of the yeast cell wall, allowing the stain to enter the cell; thus, cells with damaged cell-walls stain blue while undamaged cell do not. The problem is that if the yeast cell dies due to heat denaturization of enzyme systems and the cell wall is not affected, the dea cell will not receive the stain and the yeast cell will appear to be viable when it is not.



Yeast Culture

Yeast Culture is unique and different from the other yeast products. Yeast Culture is the only defi feed-yeast product which does not consist solely of yeast cells or yeast biomass, but, rather, is a yeast-fermented product designed to provide fermentation metabolites resulting from a specific fermentation process. By definition, Yeast Cultures contain residual yeast viability, but, they are considered a significant source of viable yeast cells or yeast biomass. The figure on the left provi a schematic representation of a commercial "yeast culture process", which illustrates how the fermentation proceeds and how the product is made.

Fermentation Metabolites:

Production of fermentative metabolites by the yeast cells, sometimes referred to as "nutrilites", is principle behind the yeast culture fermentation. A specific culture media is inoculated with live ye cells, allowed to ferment under a specific set of conditions, and then the entire fermented media i dried ... it contains both the residual live yeast cells used in the fermentation, as well as the metabolites or metabolic by-products the yeast produced. Yeast Culture is a very complex produ and contains both the "intracellular" yeast cell nutrients and the "extracellular" metabolites of fermentation. Simply feeding yeast cells will not duplicate the total "nutrient" range found in yeast culture.

When yeast cells ferment sugars, they secrete metabolic by-products ... alcohol is a good example of an extracellular metabolite resulti from yeast fermentation under brewing conditions. Variations in metabolite profile are why different fermented products (e.g., breads, beers, wines, whiskies) all taste and smell differently from each other. There are many different metabolites secreted, depending on the composition of the media fermented and the conditions of the fermentation process, including peptides, alcohols, esters and organic ac In the case of beer fermentation, these extracellular metabolites are washed away from the yeast cells and are contained in the beer a are not part of the brewers yeast. Just remember that Yeast Culture is unique among all other yeast products and must be evaluated a fermented product containing "undefined nutrients" and not based on its "known nutrient" profile like protein, vitamin, and amino acid profile.

Nutritional Yeast

The nutritional yeast products consist of yeast biomass or pure, dead yeast cells which are fed for their nutrient value. They include pri dried yeast, brewers dried yeast, torula dried yeast and whey yeast.

Yeast cells have long contributed to the nutritional value of fermented foods, like breads and beers. In some societies, "cloudy" beers a major contribution to daily nutritional needs. The cloudy sediment of yeast cells provides essential B-vitamins, minerals and amino aci And during the middle ages, infants were often fed the sediment from cloudy beer to keep them healthy and avoid nutritional deficienci

Yeasts are a good source of protein or amino acids. Approximately 40% of the weight of dried yeast consists of protein. The quality of yeast protein is excellent for a vegetable protein and it is about equivalent in quality to soybean protein. Both are rich in lysine, and are excellent supplements to cereals, whose proteins are generally low in lysine. As with other plant proteins, yeast protein is low in the sulf amino acids, but supplementing dried yeast with 0.5% methionine can raise its protein quality up to that of casein. However, there is a li to how much yeast can be fed, because about 20% of the crude protein nitrogen in yeast is in the form of nucleic acids. Nucleic acids c cause problems if over fed, because excessive nucleic acid intake results in elevated uric acid levels in the blood. High levels of uric ac tend to crystallize in the joints and in man and this can cause gout and arthritis or even renal stones.

While the nutritional value of yeast was recognized early, the identification of the nutritional factors which cured certain nutritional disea did not take place until the early 20th century. That was when the B-vitamins were discovered. Several of these vitamins were first

Primary Dried Yeast

Primary dried yeast refers to yeast (usually Saccharomyces cerevisiae or *Candida utilis*) which is intentionally grown and harvested as nutritional yeast source and is not a by-product of another industry. It is generally propagated on sugar substrates, like molasses, in aerobic bioreactors much like active bakers yeast, but dried at high temperature to kill the yeast cells. It is predominantly used in the fo industry for food enrichment and is generally too expensive for use in the feed trade

Brewers Dried Yeast

Brewers yeast is a by-product of the beer and ale brewing industry. After the beer is fermented, the yeast (*Saccharomyces cerevisiae*) i recovered from the fermentation vats, dried at high temperature to kill the yeast, and sold to both the food, health food and animal feed trades as a specialty protein, vitamin and mineral supplement.

Torula Dried Yeast

Torula yeast refers to a special yeast species (*Candida utilis*) which is often grown on the waste-water from the paper industry, called sulfite liquor. The waste liquor is high in pentose or five-carbon sugars and growing the yeast in the liquor reduces the biological oxyge demand on the waste, making it easier to dispose of – it also provides a marketable by-product. At one time, the feed industry was the primary market for Torula yeast, but now, most of it is used by food manufacturers. It got its name from *Torulopsis utilis*, which used to the yeast's name before it was reclassified by the taxonomists.

Whey Yeast

Whey yeast is yeast grown on whey lactose and consists of yeast from the species *Kluyveromyces marxianus* (formerly classified as *Kluyveromyces fragilis*). At one time there was considerable whey yeast available to the feed trade, but its availability in recent years h almost disappeared. However, as whey waste-streams become a bigger problem in the future, we may see an reemergence of whey y as a feed ingredient.

Yeast Cell Nutrients

When whole yeast cells are fed, like brewers yeast or active dry yeast, their primary nutritional contribution comes from the proteins, peptides, vitamins and minerals contained within the cell ... the intracellular biochemicals found in the yeast cell. Thus, for these nutrien to become available the yeast cell must be lysed or broken open so that the contents within the cell become available for digestion and absorption.

Gross composition of yeast biomass.	
Moisture	2-5 %
Crude Protein	50-52 %
True Protein	42-46 %
Nucleic Acids	6-8 %
Minerals	7-8 %
Lipids	4-7 %
Carbohydrates	30-37 %
Reed and Nagodawithana, 1991.	

This can happen in two ways: 1) protease and glucanase enzymes from microorganisms in the digestive tract can break open the cell via "hydrolysis" from the outside-in, and 2) the enzymes withi the live yeast cell can cause "autolysis" of the yeast cell by digesting the cell-wall from the inside-ou

When dead yeast cells, like brewers dried yeast, are fed, hydrolysis is the only way the intracellular yeast nutrients are made available. However, when live yeast cells are fed, both hydrolysis and autolysis may play a role in rupturing the cell for digestion.

As for digestibility or availability of the intracellular yeast nutrients, the nutrients from live cells are probably more available ... assuming the cell is lysed. The reason for this is heat. The higher the temperature used to dry the yeast, the more "denaturization" or destruction of the nutrients. It is not live yeast cells, per se, are more nutritious than dead yeast cells, but that dead yeasts are generally dried at much higher temperatures, which is why they are dead, and nutrient availability is generally lower.

Special Purpose Yeast Products

Irradiated Yeast

Although yeast is not a source of vitamin D activity, it does contain a sterol, ergosterol, which is converted to form vitamin D2 (ergocalciferol) when irradiated with ultra violet light. In the past, Irradiated Dry Yeast served as an important source of vitamin D activit until it was driven out of the market place by cheaper synthetic vitamin D3 (cholcalciferol) .

Selenium Yeast

Yeast is also a good source of dietary selenium. The selenium in yeast is generally in the form of selenomethionine, which is an organi form of selenium with selenium replacing the sulfur in the methionine molecule. Selenium is required for the activation of an enzyme system that has protective effects on the liver and other tissues. It appears that the selenium activated enzyme, glutathione peroxidase, prevents oxidative damage of the cell membrane and subsequent premature aging of the cell.

Brewers yeast selenium played an early role in animal nutrition, especially in pet food manufacturing. The fact that brewers yeast conta appreciable amounts of B-vitamins and selenium often accounted for its inclusion in many animal feed formulations. It was frequently u in early pet food and specialty products as a natural selenium source before sodium selenite became widely used.

Commercial "high selenium" yeasts are manufactured and sold through health food stores and sometimes added to vitamin/mineral supplement tablets. While bakers yeast may contain one or two parts per million (ppm) selenium, commercial "high selenium" yeasts ar available containing as much as 2,000 ppm selenium, 75% of which is organically bound.

Chromium Yeast

Chromium in yeast is present in the organic form called the "glucose tolerance factor" and is important in the regulation of sugar metabolism. It consists of trivalent chromium complexed with biologically active peptides, amino acids and niacin, and appears to act in conjunction with insulin to facilitate efficient metabolism of carbohydrates. It appears important for older people, diabetics, and children

trials indicate that organic chromium, either as high chromium yeast or chromium picolinate, may reduce stress in cattle and reduce fat deposition in swine.

Phaffia Yeast

Phaffia rhodozyma, known as Phaffia Yeast, is the latest yeast product to enter the feed industry. This yeast produces a red pigment u in trout and salmon feeds for its red pigmentation of the meat. This red pigment is a carotenoid called "astaxanthin". Phaffia yeast is mo expensive than the synthetic form of the carotenoid, but limited data suggests that astaxanthin from ruptured yeast cells may be a more effective pigmentor since it is in an organic matrix.

Yeast Fractions

Yeast extracts and autolysates are produced from whole yeast cells, either debittered brewers yeast or primary grown bakers yeast, an are used extensively in the food industry for flavor enhancement. Yeast extracts consist of the intracellular components of the yeast cel with the yeast cell-wall removed. Yeast autolysates consist of ruptured or lysed cells and contain both the intracellular and cell-wall fractions. Both contain 5'-nucleotides and glutamate which enhance flavor recognition. Yeast extracts are also used as microbial stimul in the fermentation industries and microbiologists use them in their laboratory growth media to optimize bacterial growth.

Yeast cell-walls remaining as a by-product in the manufacture of yeast extracts are often called yeast hulls or yeast ghosts. They consi predominantly of beta-glucans and mannans, with some chitin and protein. Yeast ghosts are often used in wine making to avoid "stuck" fermentations due to accumulating octanoic and decanoic acids. These acids are adsorbed onto the ghosts which prevents their inhibit effect on fermentation.

Autolysates:

These yeast products consist of whole yeast cells which have been broken open (lysed) by means of letting the yeast cell destroy itself using its own autolytic enzymes (autolysates), by using acids or enzymes to hydrolyze the cell (hydrolysates), or by rupturing the cell u osmotic pressure due to suspending the yeast cells in a high salt solution, called plasmolysis (plasmolysates).

They contain both the cell contents and the cell-wall of the cell. Autolysates are the cleanest of the three types of products, because hydrolysates and plasmolysates are generally high in salt or sodium content due to the salt gradients used in plasmolysis or the use of bases to neutralize the acids used to make hydrolysates.

Autolysates are used extensively by the food industry for their ability to enhance food flavors, especially in soups and snack products. enhancement is due to the yeast's nucleic acid content – the 5'nucleotides. The nucleotides add "savoriness" to food by accentuating t effects of glutamic acid or monosodium glutamate to enhance flavors.

Yeast Extracts:

Yeast extracts consist solely of the intracellular contents of the yeast cell, following lysis of the cell, with the cell-wall removed. They ar used by microbiologists in the preparation of microbial growth media and by some industrial and pharmaceutical fermentations. Extract are rich in amino acids, vitamins and trace minerals and function as growth stimulants for microorganisms.

Yeast Cell-Walls:

The yeast cell has a carbohydrate shell around it called the yeast cell-wall. It is often called a "glucan shield" and consists mostly of bet glucans and mannans. These are structural polysaccharides (chains of sugar molecules) similar to starch or cellulose. Beta-glucans ar chains of glucose sugar molecules, just like starch, but the sugars are joined together with different linkages (beta-1,3 and 1,6 linkages instead of alpha-1,4 and 1,6 linkages). Therefore, different enzymes are needed to break them down into absorbable sugar molecules. Mannans are chains of a different sugar molecule called mannose.

The yeast cell wall is thought to have a unique ability to adsorb or bind with certain things in the digestive tract, especially bad things lik toxins, anti-vitamins, viruses and pathogenic bacteria, and is presumed to have a protective or flushing effect in the gut. The mannan fraction of the yeast cell wall is also thought to be a special polysaccharide which is selectively consumed by good bacteria in the gut. These good bugs, in turn, suppress or kill the bad bacteria like salmonella. This is the same concept behind a product called fructooligosaccharide or FOS. The yeast product is similarly called "MOS", the acronym MOS standing for mannanoligosaccharide (an oligosaccharide is a polysaccharide which is only 3-10 sugar molecules long instead of hundreds of molecules, suggesting that it is a s chain mannan). When these short-chained mannans are fed, they are not digested by the animal, but are consumed by select bacteria the gut which grow rapidly and have a probiotic effect against bad bugs.

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